Cardiotoxicity: Assessing Mitochondrial Toxicity in Stem Cell-Derived Cor.4U® Cardiomyocytes

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Mito-ID® Extracellular O_2 Sensor Kit (ENZ-51045)

Mito-ID® Extracellular pH Sensor Probe (ENZ-51048)

INTRODUCTION

Mitochondrial dysfunction has been implicated in the etiology of drug-induced toxicity and of the relevant toxicities; cardiotoxicity remains the number one reason for drug withdrawal. Screening for compounds with such liabilities has been difficult due to the lack of relevant cell models and assay technologies. Axiogenesis human stem cell-derived Cor.4U® cardiomyocytes provide a homogenous, reproducible and physiologically relevant cardiomyocyte cell system that allows a specific detailed assessment of cell metabolism and mitochondrial function when combined with a suitable assay technology like Enzo's Mito-ID® Extracellular O_2 Sensor Kit and Mito-ID® Extracellular pH Sensor Probe. These assays are highly flexible 96 or 384-well fluorescence plate reader-based approaches, for the direct, real-time analysis of oxygen consumption (ETC) and glycolytic flux (ECA).

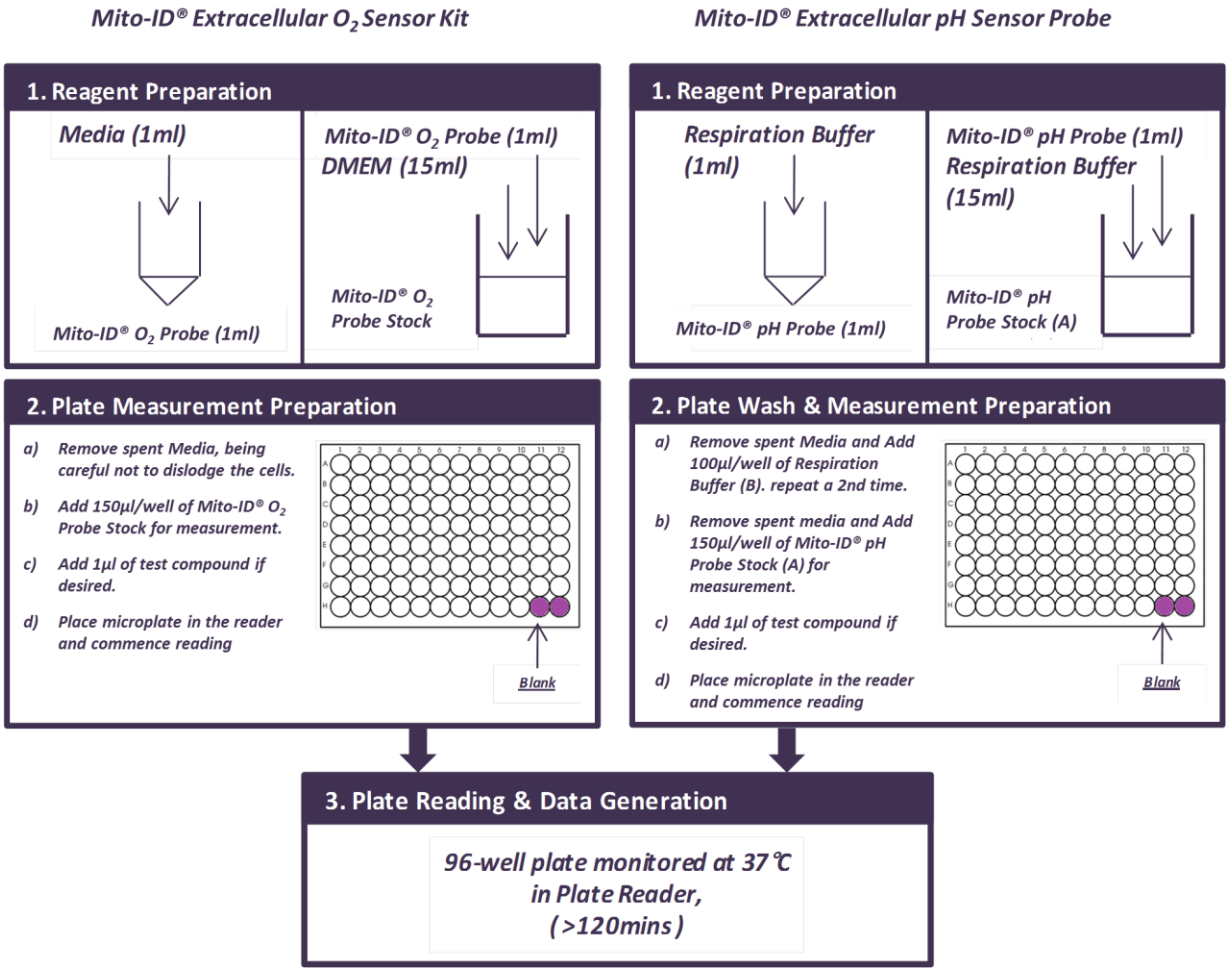
The assessment of cellular O_2 consumption is based on the ability of O_2 to quench the excited state of the Mito-ID® O_2 Sensor Probe. As the Cor.4U cardiomyocytes respire, O_2 is depleted in the surrounding media, which is seen as an increase in probe phosphorescence signal. True mitochondrial toxicity is expected to result in a decrease in oxygen consumption and a resultant increase in acidification due to glycolytic compensation while a non-specific mitochondrial insult would lead to a decrease in oxygen consumption without concomitant acidification.

METHODOLOGY

- 3-4x10^4 fresh or cryopreserved Cor.4U® cardiomyocytes were cultured for 4-5 days in fibronectin coated 96-well plates.
- Compounds were prepared in a suitable solvent typically at 15x or 150x final concentration.

Mito-ID® Extracellular O_2 Sensor Kit and Mito-ID® Extracellular pH Sensor Probe were performed as described in Figure 1.

Figure 1. Workflow



RESULTS

(**Figure 2A**) Kinetic oxygen consumption profiles of Cor.4U® cardiomyocytes are detected by using the Mito-ID® Extracellular O_2 Sensor Kit. Cor.4U® cells were seeded at 40,000 cells /well on fibronectin coated plates and cultured for 4-5 days prior to measurement. Treatments shown are FCCP (1.25µM), Antimycin A (1µM) and Nefazadone (1.56µM) versus untreated vehicle control sample, and show increase/decrease effect on O_2 consumption.

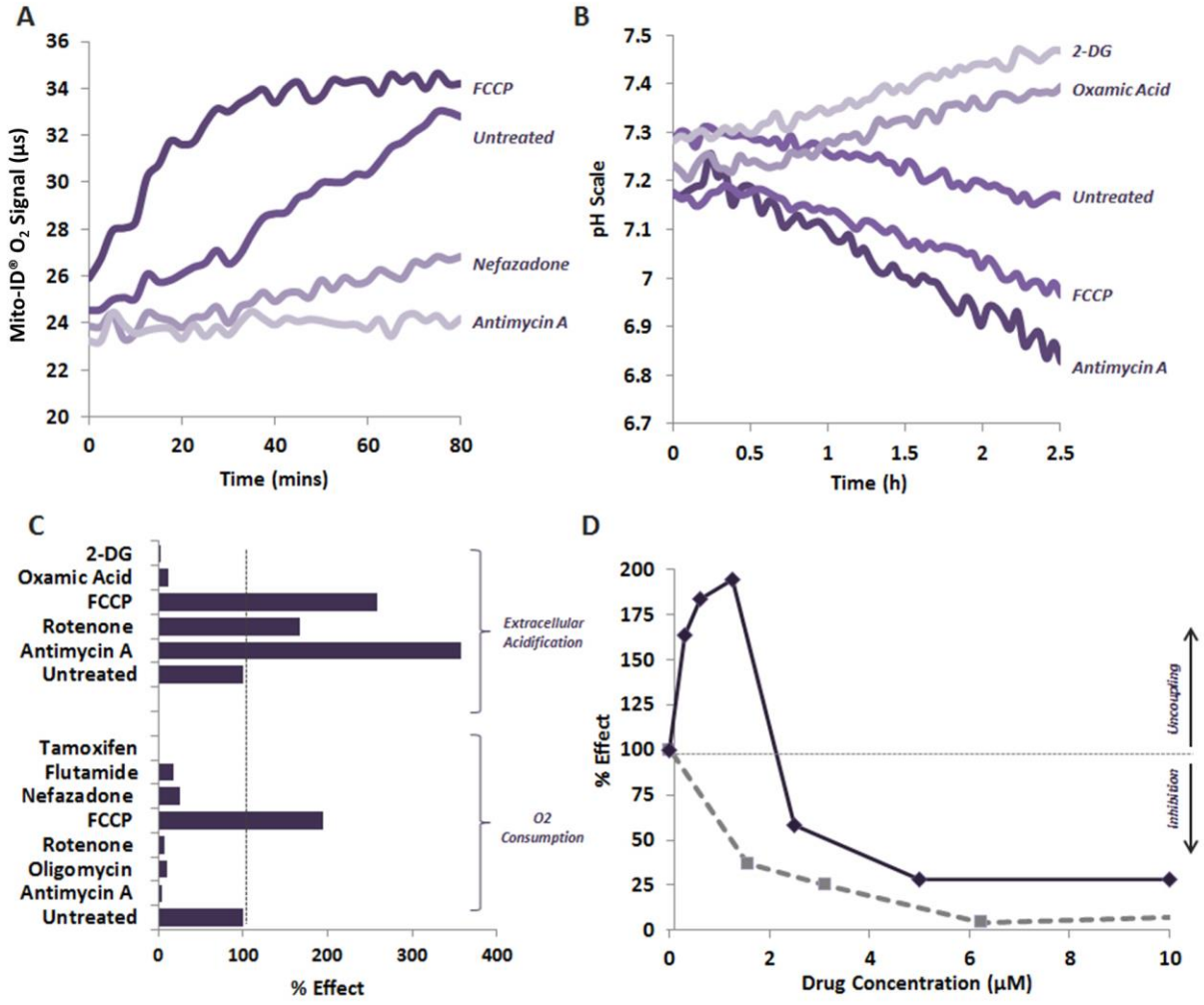
(**Figure 2B**) Extracellular acidification profiles of Cor.4U® cardiomyocytes are detected by using Mito-ID® Extracellular pH Sensor Probe. Cells were cultured as above. A decrease in pH due to extracellular acidification is evident in the untreated sample due to glycolytic flux and this flux is clearly perturbed as a result of treatment with compound. The ETC inhibitors Antimycin (1µM) and uncoupler FCCP (2.5µM) both cause increased glycolytic flux as the cells attempt to maintain ATP supply. Oxamic acid (25mM) a known inhibitor of LDH inhibits extracellular acidification rate, as expected while 2-DG (25mM) shows

competitive inhibition with available glucose and thus restricts glycolytic flux and, as a result reduces extracellular acidification.

(**Figure 2C**) Single concentration treatment of multiple drugs across both the Mito-ID® Extracellular O_2 Sensor Kit and Mito-ID® Extracellular pH Sensor Probe. The calculated % effect of the response compared to the untreated control is shown. Additional compounds were also included such as the antiandrogen, flutamide, a known Complex I inhibitor and the antiestrogen Tamoxifen, also a known mitochondrial modulator. These data again show that detailed information on the implications of drug treatment on cardiomyocyte mitochondrial function can be generated immediately post treatment.

(**Figure 2D**) Sample Dose Response Graph for the Mito-ID® Extracellular O_2 Sensor Kit treatment of FCCP (uncoupler) and Nefazadone (inhibitor).

Figure 2. Results



CONCLUSIONS

- These data illustrate the potential of stem cell derived cardiomyocytes in screening compounds for potential cardiotoxicity as they provide a homogeneous, reproducible, and physiologically relevant cell system.
- Microtiter plate based measurement of both mitochondrial function and glycolytic flux were demonstrated in a human model (Cor.4U®) using the Mito-ID® Extracellular O_2 Sensor Kit and Mito-ID® Extracellular pH Sensor Probe.
- Oxygen measurements allow the immediate detection of a mitochondrial perturbation and can distinguish between inhibitors and uncouplers.
- The combination of glycolytic flux measurements (Mito-ID® Extracellular pH Sensor Probe) and O_2 consumption measurements (Mito-ID® Extracellular O_2 Sensor Kit) provides additional confidence in the identification of mitochondrial toxicity as can be seen for Antimycin, Rotenone and FCCP.
- The value of dose response analysis is demonstrated, particularly for uncouplers whereby at lower concentrations a significant increase in oxygen consumption while at higher concentrations, non-specific cell damage causes oxygen consumption to decline.
- The combination of the human iPSC derived Cor.4U® cardiomyocyte cell model with the Mito-ID® Extracellular O_2 Sensor Kit and Mito-ID® Extracellular pH Sensor Probe provides a powerful and convenient tool for reliably investigating drug induced cardiotoxicity.